

QuantiChrom™ Alcohol Dehydrogenase Kit (DADH-100)

Quantitative Colorimetric Kinetic Alcohol Dehydrogenase Activity Determination

DESCRIPTION

ALCOHOL DEHYDROGENASE (ADH) is an oxidoreductase which catalyzes the interconversion of alcohols and aldehydes or ketones. ADH is important in humans and other organisms for the break down of alcohols which may otherwise be toxic. In yeast and some bacteria, ADHs catalyze the opposite reaction and produce alcohol as part of fermentation. BioAssay Systems' non-radioactive, colorimetric ADH assay is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is directly proportional to the enzyme activity.

KEY FEATURES

Fast and sensitive. Linear detection range (20 μ L sample): 0.4 to 80 U/L for 30 min reaction. Detection Limit of 0.1 U/L for 120 min reaction.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

ADH activity determination in biological samples (e.g. plasma, serum, urine, tissue and culture media.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 10 mL	Diaphorase: 120 μ L
NAD Solution: 1 mL	Calibrator: 1.5 mL
MTT Solution: 1.5 mL	Substrate: 1 mL 10% Ethanol

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (e.g. 25°C or 37°C).

Sample Preparation: Serum and plasma are assayed directly.

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 μ L buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at -20 to -80°C for at least one month.

Reagent Preparation: equilibrate reagents to desired reaction temperature (e.g. 25°C or 37°C). Briefly centrifuge tubes before use.

The Working Reagent (WR) is prepared by mixing, for each 96-well assay, 5 μ L Substrate, 14 μ L MTT Solution, 9 μ L NAD Solution, 1 μ L Diaphorase and 55 μ L Assay Buffer.

The Blank Working Reagent (BWR) is prepared by mixing, for each 96-well assay, 14 μ L MTT Solution, 9 μ L NAD Solution, 1 μ L Diaphorase and 60 μ L Assay Buffer (*i.e. no Substrate*). Fresh reconstitution of the WRs is recommended.

Reaction Preparation:

1. Transfer 100 μ L H₂O (OD_{H2O}) and 100 μ L Calibrator (OD_{CAL}) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 20 μ L sample into 2 separate wells. Add 80 μ L WR to one sample well and 80 μ L BWR to the other sample well. Tap plate briefly to mix.
3. Read OD_{565nm} (OD₀), and again after 30 min (OD₃₀) on a plate reader.

CALCULATION

Subtract the OD₀ from OD₃₀ for each sample and sample blank well to compute the Δ OD_S and Δ OD_B values respectively. ADH activity can then be calculated as follows:

$$\text{ADH Activity} = \frac{\Delta\text{OD}_S - \Delta\text{OD}_B}{\epsilon_{\text{mtt}} \cdot l} \times \frac{\text{Reaction Vol } (\mu\text{L})}{t \text{ (min)} \cdot \text{Sample Vol } (\mu\text{L})} \times n$$

$$= \frac{273}{t \text{ (min)}} \times \frac{\Delta\text{OD}_S - \Delta\text{OD}_B}{\text{OD}_{\text{CAL}} - \text{OD}_{\text{H2O}}} \times n \quad (\text{U/L})$$

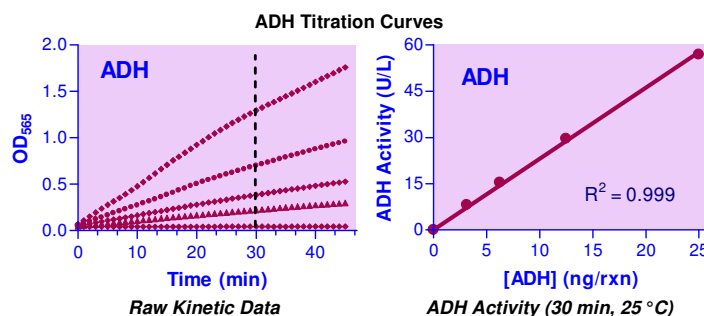
where ϵ_{mtt} is the molar absorption coefficient of reduced MTT. l is the light pathlength which is calculated from the calibrator. OD_{CAL} and OD_{H2O} are OD_{565nm} (OD₀) values of the Calibrator and water. t is the reaction time (30 min is the recommended time). Reaction Vol and Sample Vol are 100 μ L and 20 μ L, respectively. n is the dilution factor.

Unit definition: 1 Unit (U) of ADH will catalyze the conversion of 1 μ mole of ethanol to acetaldehyde per min at pH 8.2.

Note: If sample ADH activity exceeds 80 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with ADH activity < 1 U/L, the incubation time can be extended to 2 hours.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and plate reader.



LITERATURE

1. Kato, S et. al. (1984). Improved assay for alcohol dehydrogenase activity in serum by centrifugal analysis. Clin. Chem. 30:1817-20.
2. Yoshida, A et. al. (1981). Enzymatic and Structural Differences between Usual and Atypical Human Liver Alcohol Dehydrogenases. J. Biol. Chem. 256: 12430-6.
3. Theorell, H et. al. (1969). On the effects of some heterocyclic compounds on the enzymic activity of liver alcohol dehydrogenase. Acta Chem. Scand. 23: 255-60.

